

CLAIM OR CLAIMS

I/WE CLAIM:

1. A reaction mixture for performing protein synthesis reaction, the mixture comprising a prokaryotic S-30 extract combined with a supplemental mix containing buffer, salts, nucleotide triphosphates, an energy generating system, and amino acids, the reaction mixture being substantially depleted in RNase E.
2. A reaction mixture as claimed in claim 1 wherein the extract is from *E. coli*.
3. A reaction mixture as claimed in claim 1 wherein the mixture further comprises an amount of amino acids.
4. A reaction mixture for performing protein synthesis reactions, the mixture comprising a prokaryotic S-30 extract combined with a supplemental mix containing buffer, salts, nucleotide triphosphates, an energy generating system, the reaction mixture having the degradosomes substantially removed therefrom.
5. A reaction mixture as claimed in claim 4 wherein the extract is from *E. coli*.
6. A reaction mixture as claimed in claim 4 wherein the mixture further comprises an amount of amino acids.
7. A reaction mixture for performing protein synthesis reactions, the mixture comprising a prokaryotic S-30 extract combined with a supplemental mix containing buffer, salts, nucleotide triphosphates, and an energy source, wherein the reaction mixture had been fractionated by freezing, thawing and centrifugation.
8. A reaction mixture as claimed in claim 7 wherein the extract is from *E. coli*.
9. A reaction mixture as claimed in claim 7 wherein the mixture further comprises an amount of amino acids.
10. A protein synthesis reaction mixture comprising a combination of an S-30 extract and supplemental mix that has been fractionated by freezing, thawing and centrifugation.

11. An article of manufacture comprising
a fractionated *E. coli* S-30 reaction mixture which is composed of the combined
constituents of an S-30 extract and a supplemental mix combined and fractionated, the
fractionation removing RNase E from the mixture; and
a container suitable for storage and shipment containing the fractionated S-30 reaction
mixture.

12. An article of manufacture as claimed in claim 11 wherein the reaction mixture is
frozen.

13. An article of manufacture as claimed in claim 11 wherein the reaction mixture is
dried.

14. An article of manufacture as claimed in claim 11 wherein the S-30 reaction mix was
made by the process of combining an S-30 extract and a supplemental mix to make a cloudy
solution followed by centrifugation of the solution, saving the supernatant.

15. An article of manufacture comprising
a fractionated *E. coli* reaction mixture which is made by combining an S-30 extract and a
supplemental mix and then fractionating the combination, the fractionation removing most of the
DNA from the mixture; and
a container suitable for storage and shipment containing the fractionated reaction mixture.

16. An article of manufacture as claimed in claim 15 wherein the reaction mixture is
frozen.

17. An article of manufacture as claimed in claim 15 wherein the reaction mixture is
dried.

18. An article of manufacture as claimed in claim 15 wherein the S-30 reaction mix was
made by the process of combining an S-30 extract and a supplemental mix to make a cloudy
solution followed by centrifugation of the solution, saving the supernatant.

19. An article of manufacture comprising
a fractionated *E. coli* reaction mixture which is made by combining an S-30 extract and a supplemental mix and then fractionating the combination, the fractionation having the RNA degradosomes from the *E. coli* substantially removed; and
a container suitable for storage and shipment containing the fractionated reaction mixture.

20. An article of manufacture as claimed in claim 19 wherein the reaction mixture is frozen.

21. An article of manufacture as claimed in claim 19 wherein the reaction mixture is dried.

22. An article of manufacture as claimed in claim 19 wherein the S-30 reaction mix was made by the process of combining an S-30 extract and a supplemental mix to make a cloudy solution followed by centrifugation of the solution, saving the supernatant.

23. A method of making a reaction mixture for conducting a protein synthesis reaction in a prokaryotic cell free extract, the method comprising the steps of
(a) making an *E. coli* S-30 extract by lysing *E. coli* cells and centrifuging the lysate;
(b) separately, before or after step (a), making a supplemental mix including buffer salts, nucleotide triphosphates, an energy generating system, and precipitating agent that preferentially precipitates high molecular weight molecules;
(c) combining the solutions of step (a) and (b); and
(d) centrifuging the combined solutions and separating the supernatant to make the reaction mixture.

24. A method as claimed in claim 23 wherein the precipitating agent is polyethylene glycol.

25. A method as claimed in claim 23 wherein after step (c) the combined solutions are frozen and thawed prior to the centrifuging of step (d).

26. A method as claimed in claim 23 further comprising the steps of placing the supernatant into containers for commercial sale.

27. A method of performing an *in vitro* protein synthesis reaction, the method comprising the steps of

- (a) making an *E. coli* S-30 extract by lysing *E. coli* cells and centrifuging the lysate;
- (b) separately, before or after step (a), making a supplemental mix including buffer, salts, nucleotide triphosphates, an energy generating system, and a precipitating agent that acts to preferentially precipitate high molecular weight components;
- (c) combining the solutions of step (a) and (b);
- (d) centrifuging the combined solutions and separating the supernatant to make the reaction mixture;
- (e) adding a DNA template to the reaction mixture, the DNA template encoding the expression of a protein and including a promoter recognized by an RNA polymerase in the reaction; and
- (f) incubating the mixture under conditions such that protein is produced.

28. A method as claimed in claim 27 wherein the precipitating agent is polyethylene glycol.

29. A method as claimed in claim 27 wherein after step (c) the combined solutions are frozen and thawed prior to the centrifuging of step (d).

30. A method as claimed in claim 27 further comprising the steps of placing the supernatant into containers for commercial sale.